The KAT8 regulatory NSL subunit complex subunit 2 (KANSL2) is over-expressed in brain metastatic breast cancer.

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Control of gene expression includes regulation by *trans*-acting transcription factors and *cis*-acting epigenetic regulation by chemical modification of histones and the DNA^{1,2}. Brain metastases are a clinical problem in patients with breast cancer³⁻⁵. We mined published microarray data^{6,7} to discover genes associated with brain metastasis in patients with brain metastatic breast cancer. We found that the gene encoding the KAT8 regulatory NSL complex subunit 2, KANSL2, was among the genes most differentially expressed in the brain metastases of patients with brain metastatic breast cancer. KANSL2 may be of relevance to the biology underlying metastasis to the brain, and it may be of relevance as a potential therapeutic target in patients with intractable disease.

Keywords: breast cancer, brain metastases, central nervous system metastases, KANSL2, NSL complex, systems biology of breast cancer, targeted therapeutics in breast cancer.

One report described a 34% incidence of central nervous system metastases in trastuzumab-treated patients with breast cancer⁴. This alarmingly high frequency of CNS metastasis events demands an enhanced understanding of the transcriptional makeup of brain metastatic tissues to support identification of therapeutic targets. We performed a global comparative analysis of transcription in the brain metastases of patients with brain metastatic breast cancer^{6,7}. We discovered significant differential and over-expression of KANSL2, a KAT8 regulatory NSL complex subunit, in brain metastases from patients with brain metastatic breast cancer.

Methods

We used datasets GSE125989⁶ and GSE52604⁷ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 Array technology with $n=16$ primary tumors from patients with breast cancer and $n=16$ brain metastases from patients with brain metastatic breast cancer. GSE52604 was generated using Whole Human Genome Microarray 4x44K G4112F technology with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer, analysis performed using platform GPL6480. The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether KANSL2 gene expression was significantly between primary breast tumors and brain metastases in humans with breast cancer using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455).

Results

We performed global comparative transcriptome analysis of the primary and metastatic tumor tissues of patients with brain metastatic breast cancer using published microarray data^{6,7} for the discovery of novel therapeutic targets.

KANSL2 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the lysine acetyltransferase 8 (KAT8) regulatory NSL complex subunit 2, KANSL2, as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer⁶ (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to the primary tumors of patients with brain metastatic breast cancer, KANSL2 ranked 419 out of 22297 total transcripts (Table 1), equating to 98.1% differential expression. Differential expression of KANSL2 in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=6.08E-03$).

Cross-validation of differentially expressed genes across datasets in cancer can be difficult and more challenging than when cross-validating across organs and sorted cell populations due to higher levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus, we asked whether we could validate differential expression of KANSL2 when comparing brain metastases from patients with breast cancer to normal breast tissue as opposed to primary tumors of the breast. Comparison of global gene expression profiles of 10 normal breast tissues to 35 brain metastases⁷ revealed that KANSL2 was among the genes whose expression was most significantly different

transcriptome-wide between the breast and brain metastases in patients with breast cancer (Table 2). When sorting each of the genes expressed in brain metastases based on significance of difference in expression between brain metastases and normal breast tissues, KANSL2 ranked 6418 out of 41093 total transcripts (Table 2), equating to 84.4% differential expression. Differential expression of KANSL2 in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 2; $p=6.75E-04$).

KANSL2 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for KANSL2, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine magnitude and direction of change in KANSL2 expression in brain metastatic tissues. KANSL2 was expressed at higher levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast, and this difference was statistically significant (Figure 1; $p=0.0025$). We calculated a mean fold change of 1.20 ± 0.10 in KANSL2 expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 1).

Thus, by mining published microarray data^{6,7} in an unbiased and systematic fashion, we identified the KAT8 regulatory NSL complex subunit 2, KANSL2, as among the genes whose expression was most different in the brain metastases of patients with breast cancer both when compared to primary tumors of the breast and to the normal breast; KANSL2 was expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast.

Discussion

NSL, or nonspecific lethal, is an epigenetic complex consisting of the lysine acetyltransferase (KAT) KAT8, also known as males absent on first (MOF), as well as WDR5, KANSL1, KANSL2, KANSL3, MCRC1, and PHF20⁸. The NSL complex occupies the promoters of greater than 4000 genes in *Drosophila*, many of which are housekeeping genes to which the NSL complex facilitates the recruitment of RNA polymerase II⁸. One important genomic target of the NSL lysine acetyltransferase MOF/KAT8 is p53. KANSL2 contains four zinc-coordinating motifs at its amino terminus and proximal to the carboxy terminus, and a DLDV motif at the carboxy terminus utilized for WDR5 binding⁸. One unique function for the NSL complex is acetylation of lamin A/C in the nuclear envelope^{9,10}, and loss of KANSL3 results in nuclear blebbing and micronuclei formation. Interestingly, expression of K311R of lamin A is sufficient to drive chromothripsis, massive genomic instability characterized by catastrophic shattering and reassembly en masse, demonstrating that loss of lamin A acetylation by the KANSL2-containing NSL complex mimicked by K311R mutation is sufficient to drive chromothripsis, an event of largely unknown origin¹⁰. In human cancer, a genetic screen in the PANC-1 pancreatic adenocarcinoma (PDAC) cell line revealed that KANSL2 could influence invasion and migration but not proliferation in PDAC¹¹. In glioblastoma, KANSL2 expression was up-regulated at the perivascular region of tumors, associated with self-renewal, with POU5F1 controlling the expression of KANSL2; depletion of KANSL2 in GBM cells impaired their ability to form tumors *in vivo*¹².

We report here that the KAT8 regulatory NSL complex subunit 2, KANSL2, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer. KANSL2, genomic targets affected by both its acetyltransferase function and functions at the nuclear envelope in maintaining stability of the genome and integrity of the nuclear envelope may be of relevance to the biology underlying metastasis to the brain in patients with brain metastatic breast cancer, a disease with limited treatment options.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
419	221821_s_at	6.08E-03	2.9230981	-2.3055	1.20 ± 0.10	KANSL2	KAT8 regulatory NSL complex subunit 2

Table 1: KANSL2 is differentially expressed in brain metastases in brain metastatic breast cancer.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, fold change (FC) of KANSL2 expression between the brain metastases of patients with breast cancer and primary tumors of the breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
6418	A_24_P67929	6.75E-04	3.6410741	-0.85824	KANSL2	KAT8 regulatory NSL complex subunit 2

Table 2: KANSL2 is differentially expressed in brain metastases in brain metastatic breast cancer.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Figure 1: KANSL2 is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of KANSL2 in primary tumors of the breast (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean KANSL2 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and primary tumors of the breast, a *p*-value, listed above.